The Expression and Localization of Kappa Myeloma Antigen on Malignant and Normal B cells

Andrew Tasman Hutchinson

A thesis submitted in fulfilment of the
requirements for the degree of
Doctor of Philosophy

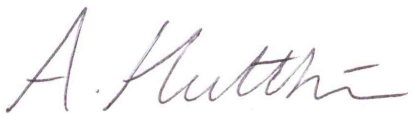
University of Technology, Sydney
NSW, Australia

August 2009

Certificate of Authorship/Originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the written preparation of the thesis, and all experimental work associated with it, has been carried out solely by me, unless otherwise indicated. Finally, I certify that all information sources and literature used are acknowledged in the text.



Andrew Tasman Hutchinson
August 2009

Acknowledgements

I would first like to thank my supervisors and good friends, Professor Bob Raison and Darren Jones. Bob was instrumental in getting my PhD off the ground with Immune System Therapeutics Ltd and I am grateful for the time and patience he dedicated to my project. He never once held me back from chasing even the most outrageous hypotheses and provided both support, and more importantly, a critical examination during the creative process. Bob, I wish you all the best for your retirement and congratulate you on your recent appointment as Emeritus Professor at UTS.

When I was an undergraduate 'wash up boy' during the early days of PacMab, Darren took me under his wing and nurtured my development as a scientist. He trained me on most aspects of working in an immunology lab, including that most invaluable technique of flow cytometry. In a wider context Darren has, and will continue to be an inspiration, having taught me the importance of science, as well as what it means to lead a balanced and 'well-rounded' life. Darren, it has been a great journey. I have no doubt this is only the beginning, and I am already looking forward to the next step!

To all past and present at Immune System Therapeutics Ltd / PacMab, what a ride it has been! It was with great perseverance that we got this antibody to clinical trial, and in this regard, the venture has been a tremendous success. Thank you for finding the funds to support my project throughout the years. I am grateful that I was given space to pursue basic research in a commercial environment. Hopefully something valuable will one day come out of it! In particular, I would like to thank Maria Lund and Dr Cameron Jennings.

Maria for being a dedicated and outstanding research assistant. Without the help of Maria, I would still be slaving away in the lab. She was especially helpful in working up the artificial membrane systems which became one of the main cores of my project. She did this with very little help, and for a time, the student became the master whilst she taught me these new techniques. Maria, it has been a great pleasure watching you develop into a scientist. Good luck with your upcoming honours year. I am sure you'll continue to excel as a researcher.

Cameron for being a great work colleague and friend. He provided me with a number of materials needed to complete my studies. Cameron, and his honours student, Vanessa Bockhorni, also performed the κLC HEK-293 transfections which were used as cellular models of KMA expression in this thesis.

Special thanks go to Paul Ramsland and Allen Edmundson - two generations of structural immunologists. For a long time my only goal was to try and reconcile my observations with what these structuralists thought was possible. Immunology, after all, performs its magic at the molecular level. Allen was an early supporter of the misfolded FLC theory. This eventually culminated into the aggregation hypothesis after a stimulating week of work in Paul's lab. Here we performed the dynamic light scattering measurements, as well as built the phosphocholine - FLC models. Thank you both for your interest and time in my project. It was a pleasure working alongside such accomplished scientists and individuals.

To my parents, family and friends, thank you for all your continued support and interest over the years. I am lucky to be surrounded by such a diverse and interesting bunch of people!

Finally, to my partner and best friend, Lucinda, you have been an invaluable source of encouragement, love and support. Thank you for all your patience. I am looking forward to a bright and happy future with you by my side.

Table of Contents

List of Figures and Tables

Abbreviations

-	negative
+	positive
°C	degrees Celsius
α	alpha
δ	delta
ε	epsilon
γ	gamma
κ	kappa
λ	lambda
μ	mu
μg	microgram
μL	microlitre
Å	angstrom
Ab	antibody
ADCC	antibody dependent cellular cytotoxicity
AIDS	acquired immune deficiency syndrome
AP	alkaline phosphatase
APC	allophycocyanin
APCs	antigen presenting cells
ATP	adenosine triphosphate
Auto-Ab	auto-antibody
Az	azide
BCR	B cell receptor
BiP	immunoglobulin heavy chain-binding protein
BJP	bence jones protein
bκFab	biotinylated κ Fab
BSA	bovine serum albumin
bVOR	biotinylated VOR

C3	complement component 3
CCDA	co-stimulatory cell dependent activation
CCIA	co-stimulatory cell independent activation
CD	cluster of differentiation
CDC	complement-dependent cytotoxicity
C-domain	constant domain
CDR	complementarity determining regions
D	diversity
Da	dalton
ddH_2O	double distilled water
D_H	hydrodynamic diameter
DLS	dynamic light scattering
DNA	deoxyribonucleic acid
DOPC	1,2-dioleoyl-sn-glycero-3- phosphatidylcholine
DTT	dithiothreitol
ECL	enzymatic chemiluminescence
ELISA	enzyme-linked immunoabsorbent assay
ER	endoplasmic reticulum
EWB	enzyme-linked immunoabsorbent assay wash buffer
EWB-T	enzyme-linked immunoabsorbent assay wash buffer with tween 20
Fab	fragment, antigen binding
Fc	crystallisable fragment
FITC	fluorescein
FLC	free immunoglobulin light chain
FκLC	free immunoglobulin kappa light chain
FλLC	free immunoglobulin lambda light chain
FSC	forward scatter
FSW	flow cytometry staining wash
g	gram or centrifugal force
GC	germinal centre
GFP	green fluorescent protein

gp41	glycoprotein 41
GPI	glycosylphosphatidylinositol
GRP	glucose-regulated protein
h	hours
HC	heavy chain
HCD	heavy chain disease
HRP	horse radish peroxidase
hsp	heat shock protein
Ig	immunoglobulin
IgA	immunoglobulin A
IgD	immunoglobulin D
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IL	interleukin
J	joining
k	kilo
kDa	kilodalton
KMA	kappa myeloma antigen
L	litre
LC	light chain
LCA	light chain amyloidosis
LCDD	light chain amorphous deposition disease
LMA	lambda myeloma antigen
LUV	large unilamellar vesicle
M	molar
mAb	monoclonal antibody
mβCD	methyl β cyclodextrin
mg	milligram
MGUS	monoclonal gammopathy of undetermined significance
MHC II	major histocompatibility complex class II

min	minutes
mL	millilitre
MM	multiple myeloma
mM	millimolar
M-protein	monoclonal protein
mRNA	messenger ribonucleic acid
MS	multiple sclerosis
MZ	marginal zone
nm	nanometer
N-Smase	neutral sphingomyelinase
OD	optical density
p	pico
PAGE	polyacrylamide gel electrophoresis
PAMPs	pathogen-associated molecular patterns
PBMCs	peripheral blood mononuclear cells
PBS	phosphate buffered saline
PDI	protein disulfide isomerase
PE	phycoerythrin
PerCP	peridinin-chlorophyll-protein complex
PLC	phospholipase C
POPC	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine
POPE	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylethanolamine
POPS	1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphatidylserine
qPCR	quantitative polymerase chain reaction
R10	RPMI 1640 media and 10% foetal bovine serum
RA	rheumatoid arthritis
RMSD	root mean square deviation
RNA	ribonucleic acid
RT	room temperature
RT-qPCR	reverse transcriptase quantitative polymerase chain reaction
RU	arbitrary response unit

s	second
SAC	*Staphylococcus aureus* cowan I strain
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
siRNA	small inhibitory ribonucleic acid
SLE	systemic lupus erythematosus
SP-A	surfactant protein-A
SPR	surface plasmon resonance
SSC	side scatter
TLR	toll-like receptor
TMNCs	tonsil derived mononuclear cells
TX100	triton X 100
TX114	triton X 114
V	variable
V-domain	variable domain
V(D)J	variable, (diversity), joining
VH	variable heavy chain
VL	variable light chain
WM	Waldenström's macroglobulinaema
XBP-1	x-box binding protein 1

Abstract

Kappa Myeloma Antigen (KMA) is a plasma membrane associated form of free immunoglobulin kappa light chain (FκLC) expressed on malignant B cells from patients with multiple myeloma (MM), Waldenström's macroglobulinaema (WM) and non-Hodgkin's lymphoma (Walker et al. 1985). KMA is recognized by the murine monoclonal antibody (mAb) mKap, and its human-mouse chimeric equivalent, cKap, which is currently undergoing clinical trials as a therapy for kappa type MM (Boux et al. 1983; Raison et al. 2005).

Earlier expression studies on KMA suggested that the antigen is not expressed by normal B cells *in vivo*. However, *in vitro* activation of tonsillar B cells induced expression of KMA on a subset of cells. Like their KMA expressing malignant counterparts, these were presumed to be FκLC secreting plasma cells or plasmablasts but, due to the lack of B cell lineage specific markers at the time, these cells were not phenotyped (Walker et al. 1985). Furthermore, given the extremely low frequency of plasmablasts and plasma cells in normal tissues, it was not possible to exclude the presence of a 'normal' KMA positive cell population *in vivo*.

The first section of this thesis expands upon this earlier work. By utilizing *in vitro* activation protocols on peripheral blood CD19+ B cells, KMA expression was induced on a subset of cells. Phenotypic analysis revealed that the majority of KMA positive cells were CD27++ CD38+/- plasmablasts and CD38++ plasma cells. Analysis from normal human tissues found that a subset of plasma cells in the tonsils expressed the antigen. These cells co-expressed CD45, indicating that they are at an immature stage of plasma cell differentiation. In contrast, peripheral plasma cells, considered to be more fully mature cells in transit from secondary lymphoid organs to plasma cell niches in bone marrow or spleen, did not express KMA. This implies that KMA expression, *in vivo*, is limited to a small subset of immature plasma cells in secondary lymphoid organs such as the tonsils.

Despite cKap's current assessment in clinical trials for the treatment of MM, very little is known about its molecular target KMA. Previous studies have showed that KMA is comprised of FκLC (Goodnow and Raison 1985); however it was never determined as to how FκLC is associated with the plasma membrane. Since FκLC is a secreted molecule, it was initially presumed that it associated with a proteinaceous 'membrane receptor' (Goodnow and Raison 1985). However membrane extraction studies, as described in the second part of this thesis, reveal that FκLC directly associates with the plasma membrane through a combination of hydrophobic and electrostatic forces to form KMA. Further investigations confirmed that FκLCs can bind directly to cellular and artificial membranes. Moreover, this binding is likely dependent on self-association processes, which suggest that KMA consists of aggregated, membrane associated FκLCs.

Lipid binding studies revealed that FκLCs associate specifically with saturated phosphocholine species such as sphingomyelin in membranes, and KMA expression was positively correlated with sphingomyelin expression in FκLC secreting cell lines.

The final section of this thesis examines how FκLCs might interact with saturated phosphocholine lipids. Molecular modeling of dimeric FκLC suggests they are able to weakly associate with phosphocholine in the conventional antigen binding pocket formed by the κLC variable domain (V-domain). Since FκLC aggregation is a feature of KMA, then the avidity effects of multi-valent binding likely increases the strength of the proposed FκLC-phosphocholine interaction. This hypothesis explains the observation of both electrostatic and hydrophobic interactions by FκLC, as KMA, with the plasma membrane - the electrostatic component, governed by single FκLC molecules interacting with the charged phosphocholine headgroups, and the hydrophobic component, due to self-association of adjacent FκLC molecules.

Finally, a model of KMA expression by FκLC secreting cells is proposed. FκLC is synthesized in the endoplasmic reticulum (ER) then transported to the golgi-apparatus and encapsulated into vesicles destined for secretion. There FκLCs interact with saturated

phosphocholine lipids, such as sphingomyelin, and undergo aggregation resulting in stable association on the inner vesicular membrane. Fusion of the vesicle with the plasma membrane during exocytosis allows for membrane associated FκLC to become exposed on the extracellular face as KMA.